Commentary

Imprinted gene expression, transplantation medicine, and the ''other'' human embryonic stem cell

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Two types of embryonic stem cells are potentially useful as sources of therapeutic material in transplantation medicine. One type (EG cells) is derived from primordial germ cells taken from the developing gonadal ridges of human fetuses. The other (ES cells) is derived from the inner cell mass of blastocyst-stage preimplantation embryos. Both types of human stem cells are capable of long-term culture and proliferation in an undifferentiated state, and both are pluripotent when differentiated *in vitro*, giving rise to a wide variety of cells from many different lineages.

Recent work on mouse EG cells (1, 2) and ES cells (3, 4) has shown that stem cell-derived tissues and/or differentiated cells (i.e., the intended source of therapeutic material for transplantation) often fail to properly control the expression of imprinted genes (those genes that are expressed from only the maternal or only the paternal allele). Given the variety of imprinting-related developmental abnormalities observed in humans and experimental animals (reviewed in refs. 5 and 6), the possibility that imprinted gene expression might be dysregulated in stem cell-derived tissues raises a potentially serious problem for human stem cell transplantation therapy.

This problem has been confronted, directly, by Onyango *et al.* (7) in this issue of PNAS. These investigators examined the expression of four imprinted genes {*IGF2*, *H19*, *SNRPN*, and *TSSC5* [also called *SLC22A1L* (see the OMIM database, MIM 602631, 3/8/2002, www.ncbi.nlm.nih.gov/omim/)]} in differentiated cells derived from three human EG cell lines. They observed transcription of only (or predominately, in the case of *IGF2*) a single allele in all informative cases. Although the investigators were not permitted to determine whether the transcribed allele was the ''correct'' allele (i.e., maternal for *H19* and *TSSC5*, paternal for *SNRPN* and *IGF2*) under the terms of their Institutional Review Board approval, their results suggest strongly that failure to regulate imprinted gene expression is not a general characteristic of differentiated cells derived from human embryonic stem cell lines. Additional evidence in favor of this conclusion was obtained by sequencing bisulfite-reacted DNA from two of the differentiated EG cell lines. Approximately equal numbers of methylated and unmethylated copies of the *H19* ''imprinting control region'' (which is normally methylated on the paternal allele and unmethylated on the maternal allele) were obtained from both lines, as expected if properly imprinted maternal and paternal alleles were present in these cells.

These results argue that general dysregulation of imprinted genes is not a barrier to the use of human EG cells in transplantation therapy. This conclusion will give comfort to one side of the social debate on stem cell research and the potential practical importance of this conclusion cannot be ignored. The research raises several additional questions that are likely to be debated in both scientific and social circles; the first is whether this was the expected result, and the second is, if it was the expected result, then why are all of the human stem cell lines on the ''research-approved'' National Institutes of Health Human Embryonic Stem Cell Registry (http://escr.nih.gov/index.html) ES lines rather than EG lines?

ES cells might not be the best source of therapeutic material for transplantation therapy.

To understand the gist of this debate, one must understand the origins of embryonic stem cells with respect to when erasure and reestablishment of imprinting is thought to occur (Fig. 1). The derivation of EG and ES cells occurs on either side of meiosis, a true focal point in the life cycle of all sexually reproducing organisms. EG cells are descended from precursors that are already on the pathway to becoming gametes, whereas ES cells are descended from precursors that are, themselves, only a few cell divisions removed from this state.

Curiously, mouse ES cells, despite their recent link to the maternal and paternal germline, appear highly unstable with respect to the epigenetic state of maternal and paternal alleles. ES cells and differentiated tissues derived from ES cells exhibit great variability in the methylation (8) and expression of imprinted genes (3, 4). These results suggest that derivatives of human ES cells might not be the best source of therapeutic material for transplantation therapy, although these characteristics must be investigated directly.

What might one predict about the epigenetic state of EG cells? Logically, maternal and paternal genome imprints must be erased and reset before maternal and paternal genomes are united in the zygote to form a new individual (Fig. 1). If EG cells are derived from primordial germ cells that have yet to erase the maternal and paternal genome imprints from the previous generation, descendents of these cells might be expected to recapitulate proper expression of imprinted genes if put into the proper cell- or tissue-type framework. If the EG cells are derived from primordial germ cells that have already erased their genome imprints, then one might predict dysregulation of imprinted genes because maternal and paternal homologues can no longer be distinguished. The practical problem is potentially simple at a conceptual level: determine when genome imprints are erased and derive EG cells before that time to ensure proper expression of imprinted genes.

From this perspective, the only thing that is clear in the discussion about when genome imprints are erased is that it is not entirely clear when genome imprints are erased. In the mouse, there is substantial variability between genes (2, 9) and between cell lines (1). Differences in methods of assessment [direct analysis of pri-

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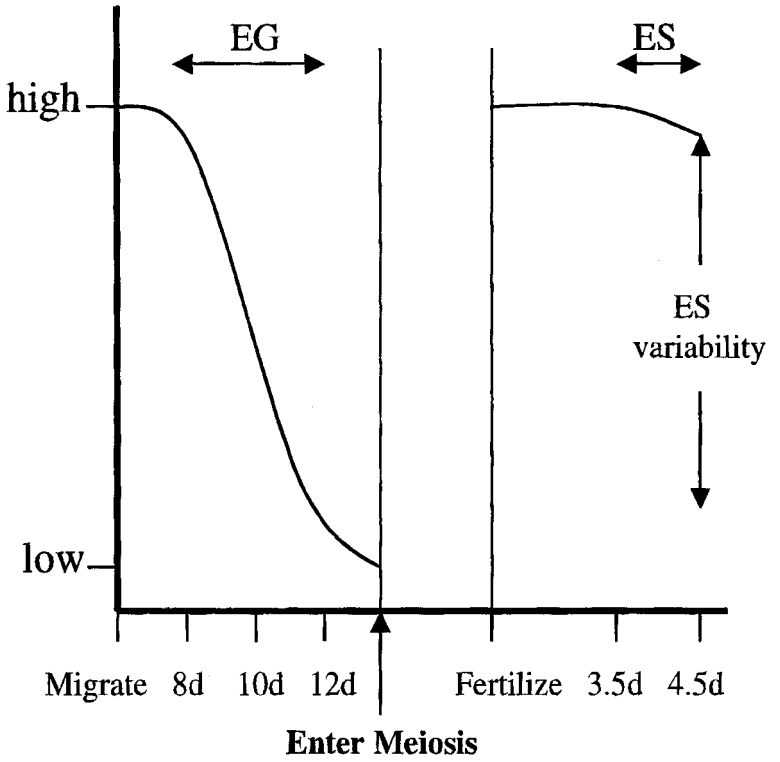

Fig. 1. Magnitude of epigenetic differences between maternal and paternal genomes as a function of germ cell development and transition to early embryonic development. Genome imprints in migratory primordial germ cells appear largely intact but distinguishing features between maternal and paternal genomes, such as differential DNA methylation, begin to be degraded by the time primordial germ cells colonize the genital ridge. Distinctions continue to disappear throughout proliferation, although some distinctions remain even as the germ cells withdraw from the mitotic cycle and enter meiosis. Different genome modifications are reestablished during male and female gametogenesis and these differences are, presumably, sufficient by the time of pronuclear fusion in the zygote to program any additional modifications that are required to result in imprinted gene expression. The *y* axis scale is arbitrary. Times on the *x* axis are for mouse development. Horizontal arrows above the graph indicate the periods at which EG and ES cells are derived. Vertical arrow at the right side of the graph represents the ''epigenetic instability'' observed in mouse ES cells.

mordial germ cells (10, 11) versus analysis of EG cell lines and chimeras (1, 2) versus analysis of embryos derived by cloning of primordial germ cells (9)] make direct comparisons between studies difficult. Analysis of imprinted gene expression in primordial germ cells provides some evidence that imprinted gene expression is largely intact in migrating germ cells (11) but is erased, or begins to be erased, shortly after the completion of migration to the genital ridge (10). Studies on mouse EG cell lines demonstrate that some distinctions between maternal and paternal alleles [e.g., differential DNA methylation at *Igf2r* (1)] begin to disappear by day 8.0, but studies on germ cells show that allelic distinctions at other loci [*H19*, for example (12)] are maintained even as cells enter meiosis. Cloning experiments with primordial germ cell nuclei indicate that imprints at several loci are progressively removed between days 10.5 and 12.5, but that the primordial germ cell population is heterogeneous for imprint erasure during this period (9). The consensus view, on mouse EG cells, is likely to be that primordial germ cells taken at an earlier stage of development (i.e., earlier than day 10.5, at most, and preferably by day 8.0) might have a better chance of maintaining imprinted gene expression than those taken at later stages.

The differentiated EG lines examined for imprinted gene expression by Onyango *et al.* (7) were derived from 5-, 6-, and 11-week-old human fetuses [these gestational ages are taken from the original description of the derivation of these lines in Shamblott *et al.* (13)]. The authors of the present report note that their results ". . . indicate an important difference in the timing of epigenetic erasure between the human and the mouse." This statement is likely to lead to some discussion over the precise correspondence between developmentally equivalent stages of primordial germ cell development in human and mouse, but it is fair to say that few would have predicted that an EG cell line could be derived from an 11-week fetus, let alone predicted that differentiated derivatives of those EG cells would maintain proper imprinted gene expression. There will be those who are quick to argue that the timing question is not settled by this result because the only informative locus in the lines derived from the 11-week fetus was *H19*, which shows the latest "erasure time" in mouse experiments. However, it is also noteworthy that all of the human EG lines (including those derived from 5- and 6-week-old fetuses) showed monoallelic expression of all imprinted genes tested and that there was little or no variability between lines. This finding is in contrast to experiments with mouse stem cells, in which the hallmark of imprinted gene expression is variability (1, 3, 4); some lines/cells show proper methylation or expression of imprinted genes, others do not.

In this regard, the report of Onyango *et al.* (7) is likely to generate some discussion because they also derived EG cells from 8.5-day mouse embryos and examined imprinted gene expression both before and after differentiation. They observed biallelic expression of *Snrpn*, *Kvlqt1*, *H19*, and *Igf2* in undifferentiated cells and monallelic expression, or highly preferential expression of one allele, after differentiation in every case. The authors conclude that ". . . the mouse EG cells examined here . . . must lie somewhere along the path of epigenetic erasure that is not yet complete."

The results of Onyango *et al.* (7) are not without character-building wrinkles, of which the most interesting may be "reversal" of imprinting of *Igf2* and *H19* in chimeras derived from mouse EG cells. Before differentiation, the imprinting control region between these two loci was found to be unmethylated on both alleles and both alleles of both genes were expressed. After differentiation *in vivo*, the *maternal* allele of *Igf2* was expressed, rather than the paternal, and the *maternal H19* imprinting control region was methylated in all three chimeras. This is not the first time that the "wrong" allele of *Igf2* or *H19* has been observed to be expressed (3, 14). But the persistent result that maternal and paternal alleles are distinguished, independently of which allele is expressed, raises suspicion that the evolutionary purpose behind the epigenetic marking of maternal and paternal alleles may involve forces other than those that act on allelic differences in gene expression (15).

Virtually all reports on human stem cell research are likely to provide fuel for heated debate in both social and academic circles, and this report is no less likely to generate controversy. But the greatest impact of this study might be to reaffirm the value of the experiment. Clearly, there is no substitute for direct inquiry, regardless of where one stands in the stem cell debate.

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